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LIVE ANTIPLAGUE VACCINE

CHAPTER VII

A BRIEF CHARACTERIZATION OF CERTAIN VACCINE STRAINS

Zhivaya Protivochumnaya
Vaktsina, Moscow, Medgiz,
1956, pp 73-85, 86-92, 99-106,
104-146

Ye. Korobkova

Live AMP Pokrovskaya Vaccine

As a result of the study of the processes of mutation of the plague microbe when influenced by various factors, especially bacteriophage, M. P. Pokrovskaya established the fact that the normal form of growth of the plague microbe is the rough form. In contrast to the majority of pathogenic bacteria with the plague microbe the rough form is the virulent one.

The plague microbe is isolated from the bodies of sick rodents and men in the rough form. On the other hand, its smooth form is ephemeral, and its appearance is accompanied by a loss of virulence of the plague microbe. According to the data of M. P. Pokrovskaya, the S-form of the plague of the mother culture. In certain cases, under the influence of bacteriophages stable smooth forms occur among the plague microbes, which are markedly distinguished from the original rough form. The acquired stable changes are transmitted by heredity. An example of such a mutation is the AMP culture, isolated and carefully studied by M. P. Pokrovskaya. This culture, according to the data of the author, arose suddenly, in saltatory fashion under the following circumstances. The culture of *B. pestis* called "Korkh", isolated in 1929 from the cadaver of a patient who had died from cutaneous-bubonic form of plague, was subjected to the effect of bacteriophage on agar. As a result, a variant was obtained which was resistant to the bacteriophage, consisting of dense, dark-brown colonies of leathery consistency, glued together by a viscous substance, and which was removed from the agar as a whole. Transplants were made of these colonies to test-tubes with agar slants. The culture which grew out was kept several days at room temperature, and when transplanted, following this, to an agar dish it gave a growth of a large number of colonies, which in their morphological character-

istics were markedly different from the culture of the phage-resistant variant as well as from the original culture of *B. petis*, "Korkh". The colonies were smooth and like drops of dew in shape. The bacteria from the smooth colonies had the shapes of large bipolar, often spherical specimens. In its biochemical properties, the smooth culture was in no way different from the original culture; it maintained its sensitivity to the bacteriophage. Serologically, it was also the same as the plague culture and agglutinated plague antiserum in high titers. The isolated culture, which received the name AMP, in contrast to the typical plague culture, turbidifies bouillon and does not form the chain which are characteristic for the growth of plague microbe in bouillon. The AMP bacteria are arranged individually, in pairs, and very rarely, chains consisting of 3-4 cells are encountered.

A distinguishing characteristic of AMP is its complete lack of virulence for the most sensitive rodents and a decreased capacity to multiply in the body of the animal. The AMP culture when injected into the body rapidly disappears, and in 48 hours (the latest, 4 days) it cannot be found at the site of the injection. In slides from the site of injection of the culture a large number of polynuclear cells is seen filled with AMP microbes.

In the infecting of animals, the culture, as a rule, localized at the site of the injection, penetrates to the regional lymphatic nodes, but does not penetrate into the blood nor internal organs.

The AMP culture is harmless to rodents: susliks can tolerate such doses as 24 billion microbes. Guinea pigs are also not very sensitive to the AMP and tolerate large doses injected subcutaneously. Further investigation have shown that the AMP culture possesses quite well expressed immunogenic properties. In experiments on guinea pigs and susliks it has been possible, by means of two and three inoculations, to protect the animals from being infected with the virulent plague culture in 80-90 percent of the cases; the mortality rate of the control animals in these experiments was from 80 to 100 percent. Attempts to restore the virulence of the strain by passage of it through the bodies of guinea pigs and susliks did not lead to any increase of the virulence of this strain.

Based on her observations on the AMP strain, its morphological and biological features, its stability, its individual range and intra-species variability, M. P. Pokrovskaya comes

to the conclusion that this strain is a new basic species in the hemorrhagic septicemia group. ✓

However, many years of study of the AMP strain in the laboratory headed by M. P. Pokrovskaya have shown that in individual cases this culture may show a certain virulence, and the animals die. Thus, a suslik which was infected by the organs of a guinea pig, which had died after being infected with a fourth passage AMP culture, died from the septic form of plague, and here, in the cultures of the organs of the animals which had died, rough colonies which rapidly transformed into smooth colonies were found along with the smooth AMP colonies. ✓

After the harmlessness of the AMP strain for animals had been established in experiments, the author injected herself subcutaneously with 500 million living microbes from a one-day agar AMP culture. Her general reaction to the inoculation was not severe, despite the fact that M. P. Pokrovskaya was suffering from influenza at the time. Her evening temperature went up to 37.8°C on the day of the inoculation and in 26 hours it reached 38.8°C, and she had a chill. The local reaction to the inoculation was expressed in the formation of an infiltrate and reddening of the skin at the site of the injection. The area of erythema, including the local reactive changes, reached 15.5 x 12 cm in size after 48 hours. The regional lymph gland could be felt 48 hours after the inoculation. ✓

All the general and local reaction phenomena lasted three days and then began to abate. ✓

After M. P. Pokrovskaya, other workers in the laboratory were inoculated with this AMP vaccine. The experiment of the vaccination was afterwards extended to include an even greater number of people. All the inoculations proceeded without any complications. ✓

At the site of the injection, lymphangitides were often noted which reached up to the regional lymphatic gland. ✓

According to the data of M. P. Pokrovskaya, the inoculations of AMP vaccine produce a leucocytosis which sets in earlier and lasts longer than the local reactive changes. ✓

The safety of the AMP vaccine was additionally and finally established by experiments on the inhalation vaccination of people with suspensions of the culture. First, M. P. Pokrovskaya tested the safety of this method of inoculations on herself. ✓

A group of persons was vaccinated by the culture inhalation method, and they tolerated such vaccination without any notable reactions of the body.

N. G. Bykov, S. G. Abramova, I. M. Zhuravlev, L. S. Kaganova and M. F. Smuter (Izvestiya Ikutskogo Protivochumnogo Instituta, Vol. VI, 1945) conducted an experiment of vaccinating 1875 persons, aged from seven to 70, with the AMP strain. The vaccinations were given once, twice and three times in doses from 500 million to 1.5 billion microbes. The vaccine was injected subcutaneously and in combined fashion--subcutaneously and by the inhalation route. After the inoculations a general and local reaction were noted which lasted for two days. The temperature in individual cases went up to 38.5-38.7°C.

The AMP vaccine has not received wide-spread distribution, since it has been shown experimentally that vaccine strains with the typical rough form of growth (KB type) protect animals from infections by virulent cultures better than does AMP vaccine. Despite this, the investigations of M. P. Pokorvskaya have exerted a great influence on the future development of the knowledge about live vaccines and on the incultations into practical use of a live vaccine. Her bold experiments on herself have given her a considerable following among the scientific-technical personnel of various laboratories.

Live ZhV *Zhukov-Verezhnikov Vaccine

*[The letters used in this case and in the preceding one in naming the vaccine are taken from the name of the originator]

N. N. Zhukov-Verezhnikov and M. M. Khvorostukhina obtained a rough form culture from the typical avirulent strain of plague microbe No 74, through the action of plague bacteriophage in bouillon culture, which rough form was markedly different from the original typical culture of plague microbe. The ZhV culture was obtained as a sudden break, and its appearance was preceded by a transition of the original rough culture No 74 into the S-form.

The ZhV strain is considerably different from the mother culture; in its cultural, biochemical properties it is similar to the causal organism of pseudotuberculosis of rodents. It

is resistant to the specific plague bacteriophage, which lyses only the plague strains in their typical growth forms, but is lysed by the phage for pseudotuberculosis; it decomposes rhamnose, does not give an agglutinative growth on bouillon, but in contrast to the typical R-form of plague microbe, the medium does not remain completely transparent but rather turbidifies slightly. On agar, ZhV grows in the form of rough, somewhat more transparent and less chromogenic colonies than the colonies of the plague microbe. The ZhV sharply distinguishes the ZhV strain from the mother culture is its capacity to grow from single cells, like the pseudo-tuberculosis microbe.

The ZhV strain is obtained in two forms: ZhV-R and ZhV-S. Its basic form of existence is the ZhV-R form. Its S-form is not stable and rapidly converts into the R-form.

The ZhV strain is very stable; subcultures of it after prolonged keeping on nutritive media and subcultures from the bodies of animals into which ZhV cultures have been injected have not once shown any tendency to pass over into the typical plague culture. During numerous investigations on extensive material, the smooth form split off from the R-form three times, but after two transplantations of it, this smooth form turned back into ZhV-R. Even with massive infections of guinea pigs with ZhV culture, ZhV-R was isolated from the animals. Serologically, the ZhV strain is similar to the plague microbe: it agglutinates anti-plague serum quantitatively. On the other hand, plague microbe is agglutinated by serum obtained from the immunization of animals with the ZhV-R culture. The ZhV strain is agglutinated by sera obtained from the immunization of animals with pseudotuberculosis cultures.

The ZhV culture is basically avirulent for guinea pigs; however, the injection of large doses subcutaneously produces inflammatory signs and necrosis at the site of the injection. With the infection of animals by moderate doses of ZhV culture, it is also found at autopsy in the internal organs: spleen and liver. However, despite the penetration of the microbes into the internal organs, no microscopic changes are seen in them, and the animals do not die. Deaths of solitary animals is recorded only after the injection of very massive doses--more than 50 billion microbes. The pathological picture, thereby, in the animals which died was not similar either to the picture of plague nor to that of pseudotuberculosis. All this gives the author basis to believe that ZhV is not an avirulent culture but rather heterovirulent, whereby the ZhV

in its smooth form is somewhat more virulent than ZhV-R. The testing of the immunogenicity of the ZhV strain was conducted on guinea pigs by means of a single immunization of them with different doses of the culture. As a control infection, such doses of the virulent culture were used which killed 100 percent not only of fresh, unimmunized guinea pigs but also guinea pigs which had been immunized by various killed anti-plague vaccines. The experiments showed the great immunogenicity of the ZhV strain.

Study of the intensity of the immunity in guinea pigs which had been vaccinated by various doses of the ZhV culture showed that the ZhV vaccine in moderate doses (from 1 million to 1 billion microbes) protects against infection by very large doses of the virulent plague culture (5000 lethal doses). With the immunization of animals by such doses of ZhV vaccine, from 77.4 to 96.9 percent survived after being infected, while 100 percent of the control animals died.

However, the great immunogenicity of the ZhV vaccine which had been established in the first experiments later began to fall off. In analyzing the reasons for this phenomenon, N. N. Zhukov-Verezchnikov comes to the conclusion that repeated growths of the ZhV strain in bacteriophage are required to restore its immunogenicity.

On the basis of all the data obtained as result of performing the experiments, N. N. Zhukov-Verezchnikov comes to the conclusion that ZhV vaccine possesses considerable immunogenicity. He considers its hetero-virulence and fluctuation of immunogenicity as the shortcomings of this vaccine. N. N. Zhukov-Verezchnikov and his co-workers have shown that by growing it in bacteriophage it is possible to increase the immunogenicity of the ZhV culture. As far as its heterovirulence is concerned, this feature conditions the ability of the ZhV bacillus to become accustomed to the body, which, undoubtedly, influences the immunogenicity of the vaccine. Practically, the ZhV strain is safe, since only massive doses, such as 40-50 billion microbes, have caused the deaths of only individual animals. It was not possible to restore the virulence of the strain or convert into the original culture by passages through the bodies of sensitive animals.

Through the investigations of N. N. Zhukov-Verezchnikov and other workers of the "Microbe" Institute, it has been established that the ZhV strain is in essence a new species, which corresponds in its basic biological features to the

causal agent of pseudotuberculosis of rodents, although in its immunological properties it is more similar to the plague microbe.

Live Vaccine No 46-S of Korobkova

The strain from which this vaccine was obtained is the smooth form of plague microbe which had been obtained through the action of plague bacteriophage on an old museum culture No 46 in the R-form of growth, under the following conditions: One loop of phage, taken from a sterile area on agar following the lysis of a normal plague microbe culture, was added to a flask with bouillon. The flask with bouillon was placed in a thermostat at 37°C for two days. After this, the bouillon, which remained completely transparent, was seeded with one loop of a two-day agar culture of strain No 46. After a day of reexamining in the thermostat, a slight turbidity appeared in the bouillon; a transplant of the turbidified bouillon to a Petrie dish with agar produced a growth of small, colorless, transparent colonies with convex surfaces and smooth borders. In smears prepared from these colonies, gram-negative, bipolar and spherical cells were found. More careful study of the newly obtained culture showed that it is the smooth form of the plague microbe, which had arisen suddenly from the original No 46 culture through the action of weak concentrations of the bacteriophage. In its cultural and morphological features strain No 46-S is very similar to AMP. It is distinguished from AMP biochemically--strain No 46-S decomposes rhamnose on the seventh day of growth. The culture obtained is lysed by all races of the plague bacteriophage in somewhat weaker fashion than the original culture. In its antigenic properties, strain No 46-S is very similar to the plague microbe and is agglutinated by plague antiserum up to 1/4 of its titer. In testing on animals, strain No 46-S proved to be completely avirulent. Comparison of its immunogenic properties with the immunogenic properties of other avirulent strains which had spontaneously lost their virulence following prolonged maintenance under laboratory conditions but which were in their normal rough forms, showed that the latter possess better protective capacities.

Culture No 46-S possesses weak invasive properties, does not spread within the body but remains localized at the site of injection no more than three days and does not penetrate any further than the regional lymph gland. Such a rapid disappearance from the body makes this culture completely harmless but

at the same time determines its weaker immunogenic properties by comparison with the EB strain. For an immunizing effect, much larger doses of microbes of strain No 46-S are required for guinea-pig inoculation than of the EB strain.

Strain No 46-S has proved to be quite stable, and it has not been possible by guinea pig passages to increase or restore its virulence. The increase of virulence of No 46-S and its reversion to the original culture may probably be accomplished only by means of its prolonged stay within an animal body. The experiment of injecting guinea pigs with a lipovaccine prepared from the 46-S strain has shown that such a possibility exists. In one guinea pig which died from ordinary pneumonia seven days after the lipovaccine inoculation, solitary colonies of the rough form, which quickly reverted to the smooth colonies grew out from the sites of injection of the No 46-S culture.

Comparative study of the immunogenic properties of the EB and No 46-S strains has shown that following the single vaccination of guinea pigs with EB cultures, they acquire a prolonged and stable immunity; under the same experimental conditions, No 46-S gives a weaker immunization. However, guinea pigs immunized with mixtures of both vaccines, which are bivalent even in small doses, showed a great degree of resistance to subsequent infection by large doses of a virulent plague culture. The bivalent vaccine of EB and No 46-S has not obtained wide-spread distribution, since it does not withstand comparison with the EV strain, which has proved to be more active.

Live Vaccine M No 74

N. N. Zhukov-Verezhnikov, T. D. Fad'yeva and A. P. Yashchuk in 1944 proposed as a vaccine strain the typical culture of plague microbe in the rough form which had spontaneously lost its virulence after being kept for 20 years on artificial nutritive media. This strain has proved to be avirulent for laboratory rodents--white mice, guinea pigs and rabbits. The guinea pigs tolerated the injection of a single agar culture of 14-12 billion microbes of strain M No 74 subcutaneously.

The immunogenicity of this culture was tested on guinea pigs and white mice. The guinea pigs were given a single inoculation of 1.5 billion microbes subcutaneously, then infected with 100 lethal doses of the virulent culture; they survived

in 100 percent of the cases. According to the authors' data, the immunogenicity of strain M No 74 was no different from the immunogenicity of the KB strain.

White mice which had been vaccinated once and twice by M No 74 strain gave a higher survival rate than mice vaccinated with the KB strain.

On the basis of their experiments, the authors believe it expedient to use the more effective, two-time vaccination, using KB vaccine for the first inoculation and M No 74 for the second.

The necessity for the creating of such vaccines stems from the fact that strain M No 74, in contrast to the KB strain, decomposes glycerine and belongs to the continental group of plague microbes, whereas the KB strain belongs to the oceanic group and does not decompose glycerine.

The authors (N. N. Zhukov-Verezhnikov, A. P. Yashchuk and one student) tested the safety of the M No 74 vaccine on themselves. The vaccine was injected subcutaneously in doses of: 1.1 billion, 600 million and 1.1 billion microbes.

The inoculations proceeded favorably and showed that the vaccine M No 74 produces a reaction which is no different from the reaction observed after the injection of other live vaccines. At the site of the inoculation a hyperemia was formed as well as a small amount of edema and moderate pain. The temperature increased within limits of 37.5-37.8°C. The regional lymphatic glands were not enlarged. In one of those inoculated a lymphangitis developed which quickly disappeared. With this work a first step has been made toward the construction of polyvalent "oceanic-continental live anti-plague vaccines" (N. N. Zhukov-Verezhnikov).

Live Vaccine of Otten

After the unsuccessful attempts to immunize guinea pigs, wild rats, laboratory white rats, mice and monkeys (*Macacus rhesus*) with various killed vaccines, Otten found that none of the killed vaccines tested protected the guinea pigs and wild rats--the animals which are most sensitive to plague infection--from being infected by virulent plague culture. On this basis Otten, like other investigators, concentrated on the study of live plague vaccine. For his experiments he

worked first with the Java strain isolated in 1920 from a bubo of a person who was sick with plague. The culture was kept on straight serum agar. In 1930, that is, 10 years after its detection, the Java culture lost its virulence for rats, but it still maintained a certain virulence. A year later, the strain lost its virulence also for guinea pigs. However, Otten preferred another culture to the Java strain which culture rapidly and spontaneously lost its virulence--the Tzhivaydezh culture [this proper name is a Russian rendition for a city in Southeastern Asia.] This culture was isolated from a rat which died of the plague, which was found in the Tzhivaydezh region (inhabited place near Bandung); hence the name of the strain. After keeping it in a test-tube in the depths of straight agar [opposite to agar slant] with serum at 50°C for four months, the indicated culture lost its virulence. Subcutaneous injection of half of the agar culture into a guinea pig and a rat did not produce any plague symptoms in these animals. It was impossible to restore the lost virulence by animal passages.

Although in the first experiments on animals, the Otten strain gave good results--with one and three inoculations it protected fourteen of fifteen experimental rats and fifteen of fifteen immunized guinea pigs--in further experiments the immunogenicity of the strain fell off sharply and it began to protect rats only in 57.7 percent and guinea pigs in 25 percent of cases. Analyzing the reasons for the drop in immunogenicity Otten established the fact that this strain began to dissociate into smooth and rough variants. (We are adhering to the terminology of Soviet authors in designating the variants). Whereas the rough form of the Tzhivaydezh strain protected rats from plague in doses from 1/5 to 1/1000 of the agar culture in 75 to 100 percent, the survival rate of rats immunized by the smooth variant fell to 24-50 percent. In experiments of using weaker doses for immunization, the effect from the smooth variants was still less favorable: the R-form protected rats in 70.8 percent of cases; the smooth variant, in 22 percent. Even in a dose of 1/100000 of the culture the rough variant protected two rats out of ten. In guinea pigs the contrast in the immunogenic properties of the smooth and rough variants was still more marked: 1/5 and 1/1000 of the R-form culture gave a survival rate of 80 to 100 percent against 15 and 30 percent obtained from the smooth variant.

In elucidating the causes for the dissociation of the vaccinal strain into smooth and rough variants, it turned out that the indicated process was only observed in individual

subcultures of the strain which were kept with inadequate care under unsuitable temperature conditions. After that, Otten used only the rough variants in his experiments, which gave a better effect. It should, however, be stated that the Tzhivaydezh strain apparently had set out on the route of intraspecies variability which leads to weakening of the immunogenicity of the culture. In this connection, it is of interest to note that the Tzhivaydezh strain began to decompose glycerine.

During the performance of the initial experiments, the minimal immunizing dose of Tzhivaydezh strain for guinea pigs equalled 10,000 microbes. In connection with the possibility of a decrease of immunogenicity of the Tzhivaydezh strain, Otten studied several other plague avirulent cultures and established the fact that some strains immunize rats better; others, protect guinea pigs to a greater degree. From this Otten draws the conclusion that in plague microbes two antigens exist: one active for rats, the other, for guinea pigs.

After the safety and immunogenicity of the Tzhivaydezh culture were studied on animals, its safety was tested out on people. Otten first inoculated himself subcutaneously with 300 million microbes and a second time, with 600 million; the inoculation proceeded without complications and did not produce any suppurations at the site of injection as Otten had feared on the basis of the fact that in animals the injection of the culture was always accompanied by the formation of an abscess. After these tests of the strain, the more extensive utilization of the vaccine for people began. Starting with 1934, the Otten vaccine began to be used on the island of Java for the mass vaccination of people.

Details on the use and effectiveness of this vaccine in people are described in Chapter VIII.

Live KB Vaccine of Girard and Robic

Girard and Robic, staunch proponents of the idea that only live vaccine can improve the epidemiological situation in a country where the fight against reservoirs of plague infection is impractical, beginning with 1932 set about the study of various strains of the plague microbe with weakened virulence. After numerous experiments the authors concentrated on a single strain, isolated in Tananarive (island of Madagascar) in 1926 from the cadaver of a person who died from bubonic plague, named KB (the initials of the patient from whom the strain was

isolated) by the authors. The virulence of the EB strain was weakened by monthly transplantations to agar at a temperature of 18-20° for a period of five year.

In its morphological, cultural and serological features, the EB strain is a typical plague strain (oceanic, according to the classification of Berlin and Borzenkov). The EB strain does not decompose glycerine, does not decompose rhamnose even after many days of keeping the culture on peptone water with glycerine and rhamnose. According to the data of Girard and Robie, this strain is avirulent for guinea pigs and rabbits by subcutaneous injections, percutaneously, conjunctivally, intranasally or intratracheally. The whole agar culture is well tolerated by these animals.

The injection of large doses into guinea pigs and rabbits intraperitoneally kills a part of the animals with signs of peritonitis, which is accompanied by septicemia; the microbes, isolated by hemoculture, possess all the properties of the EB strain, that is, they do not become more virulent. As far as white mice and rats are concerned, the high sensitivity of these animals to the toxin does not permit them to tolerate large doses of the EB strain, whereas rats (*Rattus rattus*), which are less sensitive to the toxin, tolerate the EB strain as well as the guinea pigs.

Afterwards, new facts attracted the attention of the French authors. In their combined work with the Bablet it was established that the injection of 1 billion EB microbes intraperitoneally into guinea pigs produces in them a number of reactive phenomena specific for this strain. Usually, in killed animals, beginning with the fifth day after the injection of the microbes, an increase is found, at autopsy, of the size of the spleen, the dull, rough surface of which was most often covered with nodes which were sharply distinguished by their gray color against the dark-violet background of the organ. On the 15th day, these nodes were diminished, and they disappeared altogether as seen on the 20th-45th day.

On histological examination of the preparations of the spleen from the guinea pigs which were killed from the fifth to the 30th day after the injection of the EB strain, this nodal reaction is noted even in the absence of microscopic changes. Similar nodes appear also on the liver, but much less often. The reaction described is seen also in animals which had been infected by massive subcutaneous doses. Such a reaction does not occur either after the injection of killed vaccines or of live cultures with very weak or non-existent

immunizing properties. The authors believe that the changes noted in the spleen are of importance for the control of the immunogenic capacities of the strain.

On the basis of their experiments on the study of the vaccinal strains of the plague microbe, Girard asserts that the effectiveness of live vaccine which protects actively against the plague is not determined only by the content of live microbes in it. The presence per se of live microbes in the vaccine does not characterize its immunogenicity. The immunizing properties of the live vaccine are the functions of the residual virulence of the strain. "Weakened virulence does not mean absence of virulence" (Girard).

It is practically exceptionally important that the passages of the EB culture through the organs of animals do not lead to an increase of the virulence of it. Nor has a return of virulence been seen in microbes of the EB strain isolated from abscesses arising at the site of injection of the massive doses of EB microbes subcutaneously (Ye. I. Korobkova).

For the study of the immunogenic properties of the EB strain, Girard and Robic utilized only guinea pigs--animals which are most sensitive to plague and which are more difficult to immunize than others.

According to the data of Girard and Robic and Ye. I. Korobkova, guinea pigs vaccinated by the EB strain, survived in 100 percent of cases following a control infection by large doses of a virulent culture; a strong and lasting (up to 13 months) immunity is created. The guinea pigs can be particularly well immunized by single injections of EB culture subcutaneously, percutaneously, by installation into the eye, or into the nose. The immunized guinea pigs become resistant to bubonic and primary pulmonary plague. An immunity is elaborated to the natural infection by the bites of fleas infected with plague.

In 1932, Girard and Robic set about the testing of their strain on people. The first experiments were performed on several lepers and on one of the co-authors--Robic. The injection of small doses of EB were not accompanied by any complications. After this, another 90 lepers were inoculated and 3 volunteers. The reaction to the injection of the EB microbes was expressed in an erythema and puffiness of the skin around the site of the injection of the vaccine. The reaction was not accompanied by any notable change in the lymphatic glands.

The temperature in a part of the persons inoculated went up to 38.5-39°C and remained there for 24 hours. After performing additional experiments on volunteers, whereby one of the physicians inoculated himself with an entire agar culture (this bold experiment terminated favorably), the work of a more extensive vaccination of the population was begun.

Live MP-40 Vaccine of Kasuga

In 1938, Miskuchi isolated a culture of the plague microbe from the spleen of a human cadaver dead of the plague. The virulent culture obtained behaved like a typical continental strain variant in its morphological and cultural features. On agar, the microbes formed a well expressed capsule at a temperature of 37°C. This Miskuchi strain was the original from which Kasuga obtained his vaccinal strain under the following conditions. A suslik was infected with the Miskuchi during the period of its hibernation. Eleven days after this hibernation, the suslik woke up and over the course of 14 days appeared healthy. After this, the suslik was killed, and from its inguinal and axillary lymph nodes a culture was isolated which did not form capsules at 37°C. This non-capsulated strain was designated MP-40 by Kasuga. In all its other features it was in no way different from the original culture.

Strain MP-40 was passaged 28 times through guinea pigs which were injected with 1/10000 of the culture, but the ability of the microbes to produce capsules could not be restored. After this, Kasuga passaged the MP-40 strain through guinea pigs which had first been immunized by 300 million microbes of live EB vaccine.

In addition, he passaged the strain for a long time (15-20 times) on bouillon containing 10 percent alcohol at a temperature of 40°. As a result, Kasuga obtained a stable, non-capsulated avirulent immunogenic strain of MP-40.

According to the data of Kasuga, the vaccinal strain could be obtained from the virulent culture by means of passaging it through an immune body with subsequent cultivation of the microbes on bouillon with 50 percent serum. With this method of culture of the strain, a non-capsulated culture could be isolated as early as five days. At first, capsulated microbes were found along with the non-capsulated ones, but after 25 days of keeping the strain on this medium,

only a few non-capsulated forms of microbes began to be isolated. According to the data of Kasuga, who used this method, non-capsulated, avirulent microbes possessing immunogenic properties could be obtained readily. The greatest degree of immunity was obtained in guinea pigs after vaccination of them with mixtures of EB vaccine and MP-40 vaccine.

In 1940, MP-40 vaccine was first inoculated into 18,890 persons in 96 inhabited places; another 50,000 persons were later inoculated with the same dose. The effectiveness of the immunization performed was checked by Kasuga and Iwanaga in 1941. In evaluating the effectiveness of the killed and of the live vaccine, the authors believe that the killed vaccine lessens the mortality rate among those who become ill with the plague. Inoculations of the live vaccine not only lessen the mortality rate among those who fall ill but also, in some of the cases, protect against the disease.

Of those inoculated with the killed formalin vaccine, 15 percent of those who become ill recovered; of those inoculated with EB vaccine, 35 percent recovered; of those inoculated with MP-40 vaccine, 40 percent recovered.

Second inoculations of the killed vaccine did not exert any influence on decreasing the mortality rate among those who became ill. However, the double inoculation with live EB and MP-40 vaccines (successively--first EB, then revaccination with MP-40) led to the recovery of all those afflicted who had been vaccinated.

CHAPTER VIII

EPIDEMIOLOGICAL EFFECTIVENESS OF LIVE ANTI-PLAGUE VACCINES

The decisive criteria of the prophylactic value of live vaccine are the observations of its epidemiological effectiveness. Inoculations against plague by killed vaccines, which has been practiced for many years on the islands of Madagascar and Java, have not notably effected an improvement of the epidemiological situation and have not assured personal prophylaxis. Cases have been seen also in persons who have been repeatedly vaccinated with the killed vaccines at intervals of two to three months. By virtue of this, the inoculations ceased to satisfy the sanitary organizations and were censured by the population itself, which, not seeing any benefits from the inoculations, stopped being vaccinated; therefore, the use of the killed vaccines was actually stopped there.

The verification of the effectiveness of the live vaccine on the island of Java and many other foci on the basis of a decrease in the morbidity rate proved to be exceedingly difficult in practice. Even when such a vaccination is performed where one half of the members of a family is inoculated and the other remains uninoculated, a situation is possible where some are exposed to a greater danger of plague infection than others. In view of this, the decrease in the mortality rate from plague among those who had been inoculated and those who had not been inoculated began to be reckoned for determination of the effectiveness of the vaccine.

Having convinced himself of the high degree of effectiveness of live vaccine and of its safety in experiments on animals and on volunteers, Otten introduced the live vaccine proposed by him into the anti-plague practice on the island of Java. Beginning with 1934, the inoculations were given in two districts: Banjaran and Batojajar. In all, 37,500 persons were inoculated. In giving the inoculations, the so-called alternative method of vaccination was used, whereby half of the members of each family were vaccinated, while the second half remains uninoculated and serves as a control. The vaccine was injected once in a dose of three billion microbes for adults and about 1.5 billion microbes for children. The inoculations produced a moderate reaction. During an outbreak of the plague

there were many cases of the pulmonary form which complicated the recording of the results, because, according to the assertion of Otten, his vaccine, or those doses which he used, were inadequately effective protection against pulmonary plague.

The morbidity rate among those inoculated and those not inoculated were not taken into account; only mortality rate was taken into account. After the epidemic was over, it was established that 14.6 percent had died among those who had been vaccinated; 85.4 percent, among those who had not been vaccinated.

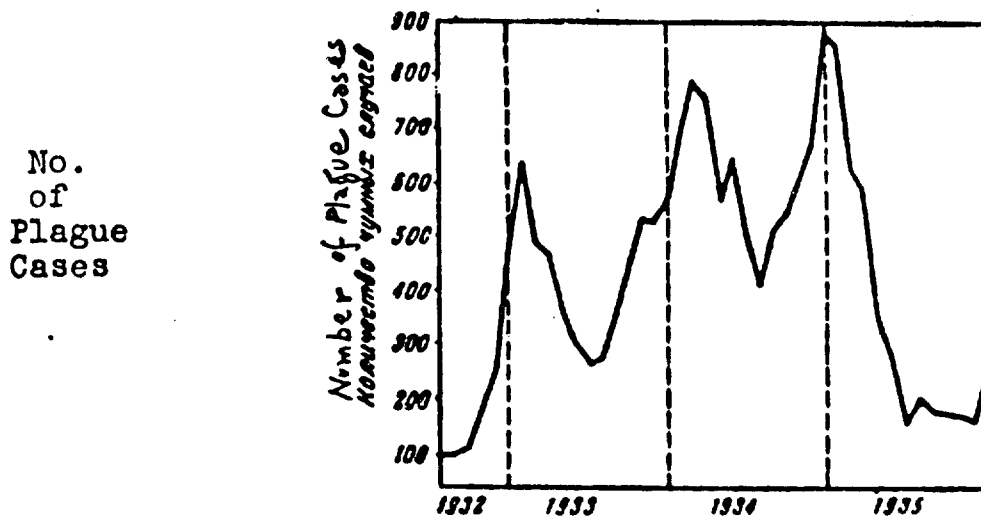


Fig 10, Influence of inoculations of the population with the live vaccine of Otten on the course of plague infection in Bandung (Island of Java) from July 1932 through December 1935. Inoculation were begun in 1934.

In the Batojajar district there were fewer cases of pulmonary plague, the form which decreases the effectiveness of the inoculations. In this district in general the vaccination decreased the mortality rate from plague by ten times in comparison with the mortality rate among those who had not been inoculated.

The large-scale vaccination conducted on the island of Java in 1955 led to the considerable decrease there of cases of plague. In all, 2,082,261 inoculations were given on Java. Almost 90 percent of the basic population was inoculated. From the time of starting the vaccination on

masse, December, 1935, through July, 1936, the morbidity rate from plague began to decrease notably.

According to the data of Otten, the inoculations with live vaccine given to 3,000,000 persons demonstrated their complete safety and significantly greater effectiveness than the killed vaccine inoculations. The curves of morbidity rate with plague by years, taken from the work of Otten, very graphically depict the gradual decrease in mortality rate from plague on the island of Java after several campaigns of live vaccine inoculations had been conducted there (Fig. 10).

In 1939, the number of cases decreased to one fourth of the number in the preceding years.

According to Otten, the immunity conferred by his live vaccine lasts no more than six months, after which a re-vaccination should be given.

Despite the satisfactory results obtained on Java from the immunoprophylaxis of plague with live vaccine, Otten believes that the basic prophylaxis of plague is a system of measures directed at the extermination of rodents and their fleas, at the improvement of living, especially housing, conditions of the local population.

Girard and Robie, Devignat and others using EB vaccine, present better indices of effectiveness of live vaccine.

From December, 1932 to January, 1934, 13,000 persons were inoculated on the island of Madagascar. Continuing into 1934, another 96,000 persons were vaccinated. Systematic examination of all persons who had been vaccinated in the entire sector gave the authors the right to assert that the EB vaccine is safe. As far as the effectiveness of it is concerned, without being absolute figures, it exceeds by many times the effectiveness of the killed vaccines which had been in use for ten years on Madagascar.

Beginning a new campaign of vaccination, the authors were guided by the principle of massiveness of the scale of inoculations in the hope that the plague morbidity rate would decrease with the increase in numbers of those vaccinated.

The comparison between groups of those inoculated and control groups, therefore, was secondary. From November, 1935 to October, 1938 a total of 2,443,405 persons were inoculated and revaccinated. All the inoculations proceeded

completely favorably. The curve of the plague morbidity rate dropped sharply. The number of cases of plague dropped from 3035 in 1936 to 637 in 1938 (Fig. 11).

As indices of the results of the campaigns conducted, Girard and Robic presented material on the vaccination of three inhabited places. In one place, where up to 220,000 inhabitants were counted, 209,000 were inoculated with EB vaccine, which comprised 95 percent of the entire population.

During the epidemic period, 160 cases of plague were registered, or 70 cases per 100,000 persons. In two adjacent inhabited places with 135,000 inhabitants, no inoculations had been given. There, 426 cases of plague were found, which comprised 315 cases per 100,000 inhabitants.

In the Ambotalampu district, 23 cases of plague were found among 6,306 persons vaccinated and 38 cases among 15,000 uninoculated, which comprises 23 cases per 100,000 inhabitants in the inoculated group against 253 cases per 100,000 population in the uninoculated group.

In five large areas, the following results of vaccination were obtained for 1937-1938:

TABLE

No of area	Number of cases of plague per 100,000 inoculated persons	Number of cases of plague per 100,000 non-inoculated persons	Decrease of Morbidity rate
1	8	35	4.3 pasa
2	18	84	4.9
3	34	376	11
4	27	208	7.7
5	34	101	3

As seen from these data, the morbidity rate among the persons inoculated on the average dropped by six times compared with the morbidity rate among those not inoculated.

1933-1934 1934-1935 1935-1936 1936-1937 1937-1938
 3493 cases 3605 cases 3035 cases 1376 cases 596 cases

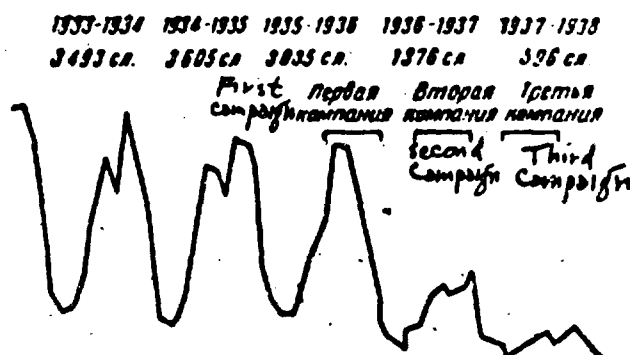


Fig. 11. Course of plague endemia and epidemia on Island of Madagascar as a result of inoculation of the public with EB vaccine from November 1935 to December 1937.

The curve of the morbidity rate on the island of Madagascar, beginning with 1921, when 187 cases of plague were registered, continued to increase steadily in subsequent years; in 1933-1934, 3493 cases were counted. In subsequent years, thanks to the vaccination of the population en masse by live vaccines, the morbidity rate declined, and in 1940 it decreased to 200 cases.

Girard points out that when the entire population in the focus is included in the inoculations, the epidemic stops. The mortality rate among those vaccinated who became ill decreased by 80 percent. The fact noted by Girard deserves attention that in those who have been vaccinated, plague is not accompanied or is rarely accompanied by secondary pneumonia, and also that septicemias are rarely observed in them. These data, understandably, are of great epidemiological significance.

Despite the demonstration of the decrease in mortality rate from plague on Madagascar by the figured data, the following questions arise: to what extent is this decrease conditioned by the inoculations, what is the role of the vaccine in the improvement of the epidemiological circumstances, did not the decrease in plague mortality rate come from the influence of natural conditions?

India and Senegal present good examples in this connection.

Is not the result of vaccination on Madagascar simple coincidence?

To this argument the French authors bring forth the following observations and facts, which argue quite heavily in favor of the significance of the live EB vaccine in the decrease of the plague morbidity rate on Madagascar:

1. The virulence of the plague microbes isolated from people and rodents had not been weakened, which was evidenced by the laboratory tests of these cultures.

2. Plague epizootics in the mouse-like rodents were found yearly, just as before.

3. The census of *Xenopsylla cheopis* fleas had not diminished.

4. The fight against rats and their was not conducted there, as it had been before.

5. Plague in man appeared in all of its three clinical forms: bubonic, septic and pulmonary.

The data of 1948 confirm the fact that by virtue of the large-scale inoculations of the population with EB vaccine the plague morbidity rate in Madagascar remains low, as before.

The data of Le Gaulle on plague in Madagascar are of interest. Plague pneumonia is particularly widely spread there in the areas of the high plateaus and is conditioned by the climatic conditions--low temperature in these places (lower than 15°). The mortality rate from plague there reaches 9.4 percent, and this figure has actually been lowered, judging by the diagnoses of plague at autopsies.

Before the inoculations of EB vaccine, from 1930-1934 cases of plague per 10,000 population were successively by years: 13.1--28.9--30.4--26.4--25.8. From 1937-1941, when the inoculations with live EB vaccine were extensively used, the morbidity rate decreased successively by years to: 6.6--4.5--4.7--5.2--1.8. In conclusion, the author expresses the hope that human plague, like smallpox will disappear on the island of Madagascar.

There is basis to believe that the inoculations with live vaccine, even if they cannot altogether eliminate endemic plague, because a reservoir and keeper of the

plague exist-- the local rodents and their ectoparasites, still can decrease considerably the threat of epidemic outbreaks. Beginning with 1944, the use of the live plague vaccine as an anti-epidemic measure began to spread more and more into other countries.

According to the data of Devignat, in the Belgian Congo (Lake Albert) systematic rat-elimination and insect-elimination has not led to any notable decrease in the number of rats and fleas in the dwellings of the local population. The most effective prophylactic measures are the inoculations with live EB vaccine, which give a decrease of morbidity rate of $7\frac{1}{2}$ times there. Rat elimination and examination of rats are conducted for establishing the presence of epizootics among them, which is the signal for the start of an inoculation campaign.

Devignat presents the results of inoculations with EB vaccine in six inhabited places located on the western shore of Lake Albert-Niansa. The inoculations were used exclusively in those inhabited places where plague had been found in people, rats and fleas. In such foci, 16 cases of plague were registered among 400,000 inoculated persons; during the same period, 116 persons fell ill with plague among those in the same group who had not been inoculated. The EB vaccine protects against the glycerine-positive variant of the plague microbe, which is the causal agent of plague in the Belgian Congo.

In South Africa, plague of wild rodents is an important problem--the enzootic focus is continuously expanding. Vigorous measures were taken for the inculcation of the use of live plague vaccine prophylaxis. A vaccine of the K-120 South-African strain and the EB vaccine were used. Both strains were used in concentrations of one billion microbes per cc. The vaccine was injected subcutaneously in a dose of 1 cc.

The immunization of 40,000 persons was very effective, despite the fact that the reaction was weak in those who had been inoculated. Observations of 14 outbreaks showed that among 24,000 inoculated only seven cases of pulmonary plague occurred, seven cases of bubonic plague and one leptic case of plague. In the latter case of plague, the patient recovered. The resistance after vaccination begins on the fifth day and reaches its maximum on the tenth day.

According to the data of Peary and Grasse, the South-

African avirulent strain of plague microbe K-120 is in its immunogenic properties similar to the Otten strain--in its capacity to immunize rats. The EB strain is less active for rats, but immunizes guinea pigs to a greater degree.

Based on the fact that the reaction of man to these two immunological types has still not been established, Grasse (1940) suggested using the vaccine made from a mixture of two strains: the Tzhivaydezh of Otten and the EB of Girard. The inoculation dose was one billion microbes. In the beginning of 1940 in the Institute of Johannesburg (South Africa) a vaccination of volunteers was performed. In the following year, during an outbreak of plague 1000 persons of the local population and 50 Europeans were inoculated with this vaccine in a single village. As a result of these inoculations, among 110 vaccinated persons who had been in close contact with patients with the pulmonary form of plague, six persons became ill with pulmonary plague, and five of them died. These five persons had been in contact with patients on the first day after vaccination. In another area, 6 persons were inoculated who were then in contact with patients with pulmonary plague. Following the vaccination, not a single case of plague was registered among them.

The reaction to the injection of such a bivalent vaccine was moderate; the temperature remained within the limits of normal.

It should be noted that up to the present time, according to the data of foreign authors, the vaccine strain EB, especially EB-76, is the most stable and most effective of all the vaccine strains used for immunoprophylaxis of plague.

At the same time, observations have been accumulating in recent years attesting to the weakness of the EB vaccine in a number of places. This calls for a reexamination of the vaccine strains as well as of the methods of using them.

CHAPTER X
CONCERNING THE POSSIBILITY OF PROTECTION AGAINST PULMONARY
PLAGUE BY LIVE VACCINE

At a conference in Mukden (1910), in the evaluation of the various methods of protecting people against plague the desire was expressed in the form of a resolution to test out for inoculation purposes, live plague cultures which had been weakened in their virulence. At this same conference the suggestion was made of performing experiments of infecting animals (guinea pigs, white mice and monkeys) through inhalation with the aim of determining a better means of vaccination against pulmonary plague. Since that time more than 40 years has passed. During this period of time the immunology of plague has achieved great progress. Live anti-plague vaccine has entered solidly into the system of measures for fighting against plague. However, the question of methods of protection against pulmonary plague still remains of current importance.

Epidemiological data attest to the fact that it is possible by means of live vaccines to create a considerable resistance to infection by bubonic plague; the latter has been demonstrated in foci where plague has a particularly severe character (Madagascar, Java, Uganda).

However, the resistance of those inoculated against primary pulmonary plague is inadequate. The results of the inoculations which were given on Java and partly on Madagascar make us believe that the live vaccine, or those doses and methods of vaccination which were used there for vaccinating people, protect only weakly against primary pulmonary plague, although in principle active live vaccine should protect against all the clinical forms of plague, since the connection between these forms is very close. It is quite widely known that secondary pneumonia or even marked pulmonary congestion, which is a common symptom of the terminal phase of fatal bubonic plague, can produce primary plague pneumonia among contacts of the case and become the cause of an outbreak of pulmonary plague which is spread by means of direct transmission from man to man.

Plague bacteria which are isolated from a bubo or from the phlegm of patients with primary pulmonary plague are identical, and depending on the method of infection produce the bubonic form of plague when injected subcutaneously or

intracutaneously and the pulmonary form when injected directly into the respiratory tracts.

In the laboratory to date it has not been possible to confirm hypothesis, which is supported by certain investigators, that plague microbes isolated from patients with the primary pulmonary plague possess some kind of special tropisms for the lungs. Because of this, the point of view of individual authors that it is impossible to create resistance to pulmonary plague by means of parenteral injection of the live vaccine seems to be without basis.

The view that it is impossible to create an immunity against the pulmonary form of plague is entirely justifiable when based on the results of vaccination with killed vaccines, which have been inadequately effective even for protection against bubonic plague.

As far as the live vaccine is concerned, the possibility of protecting guinea pigs under experimental conditions has been proved, which had been inoculated once with a large dose of the vaccine subcutaneously, against primary pulmonary plague produced by the serogenic route of infection--the method of inhalation of a virulent culture (Ye I. Korobkova and A. N. Kraynova, 1939). Under these experimental conditions, three nasal vaccinations protected against infection subcutaneously by a virulent culture and protected more weakly against infection by the intranasal method (table 1).

Table I

Immunization against pulmonary plague							
Immunization Method	Vaccine Dose	Inter- vals	Inocula- tion Dose	Inocula- tion Method	No of guinea pigs	Died	Survived
Hypodermic single-shot	2,000,000 microbes	--	25 mill. microbes	Nasally	10	0	10
The same	2,000,000 microbes	--	25 mill. 250 mill. in suspension	Inhalator	12	0	12

Nasally triple- shot	700 mill microbes each	3 days	25 mill. microbes	Nasally	10	1	9
Control	--	--	25 mill. microbes 25 mill.	"	9	9	0
Same	--	--	250 mill. in sus- pension	Inhalator	9	9	0

Girard obtained the same results with intratracheal infection of guinea pigs which had been immunized subcutaneously. Based on his observations of inoculated people and of experiments with guinea pigs, he came to the conclusion that the effective live EB vaccine can protect against all the clinical forms of plague.

Observations show that in individual guinea pigs which had been immunized by live vaccine and which died a long time after the infection, there is very often found involvements in the lungs in the absence of macroscopic changes in the spleen and other organs. Guinea pigs and white mice which have been inadequately immunized against plague microbes at the site of the injection and regional lymphatic glands in the presence of the inadequate resistance of the body creates the conditions for the penetration of the microbes through the blood into the pulmonary tissue and the formation there of pneumonic foci.

These observations have served as the basis for the conclusion that for protection against experimental pulmonary plague of laboratory rodents, and therefore also of man, the live vaccine should be administered directly into the respiratory tracts, because with all the other methods of immunization the lungs remain inadequately protected.

At the present time, there have arisen two trends in the problem of methods of protecting against pulmonary plague.

Some investigators believe that the live vaccine used subcutaneously, percutaneously and intracutaneously and even nasally can confer a stable immunity upon the body against all the clinical forms of plague--bubonic, pulmonary and septic--under the condition that the vaccine strain

possesses good immunogenic properties. All the organs and tissues regulated by the nervous system acquire resistance to the plague.

Other investigators are inclined to believe that for protection against primary pulmonary plague the vaccine must be administered directly into the respiratory tracts--nasally, by the inhalation method, etc.

Recently, the need for immunizing the respiratory tracts has been motivated by the fact that pulmonary tissue possesses a so-to-speak weakly developed reticulo-endothelium and has little permeability for antibodies (M. P. Pokrovskaya and L. S. Kaganova).

It should be mentioned that the proponents of the latter trend nevertheless recognize that for protection against pulmonary plague the vaccinations through the respiratory tracts should be combined with vaccine inoculations subcutaneously (M. P. Pokrovskaya).

The idea of immunizing through the respiratory tracts arose after it was established that the subcutaneous inoculations with killed vaccine do not protect against pulmonary plague. In the development of these ideas the experiments and observations of Bazarov (1899) played a special role.

According to the data of Bazarov, the implantation of plague infection in the body is possible through all the mucous membranes. The mucous membrane of the nose is the most sensitive, then comes the conjunctiva of the eye, mucous membrane of the mouth, etc. When material containing plague microbes was applied to the mucous membranes of the noses of guinea pigs, rabbits and white mice, plague pneumonia developed in them.

In the course of his experiments. Bazarov made the observations that in guinea pigs which had been immunized by killed vaccine, the changes which occurred in the lungs after the nasal infection by a virulent culture were considerably more marked than occurred in the control animals.

On this basis, Bazarov came to the conclusion that in plague a general immunity may be attained if all the organs are immunized, since the various organs are not equally sensitive to the plague infection. "The spleen and the liver acquire immunity first," he said, "whereas the lungs

are in last place@.

It should be emphasized that Bazarov used killed vaccines for the immunization of animals, which vaccines are incapable of producing a high degree of immunity either against pulmonary plague nor against the bubonic form.

In the body there are tissues and organs, undoubtedly, which are more sensitive and less sensitive to the infective nidus, or, more accurately, which react more strongly or more weakly to the implantation of the plague microbe.

The view, however, that in the body there exist as many immunities as there are sensitive tissues, or in other words, that each organ has its own immunity, is completely without basis and unacceptable.

Such a view contradicts our concepts of the integrity of the body and the leading role of the nervous system in all the processes of the body.

Bazarov drew the entirely correct conclusion that killed vaccines do not protect against pulmonary plague. His conclusion was based on the infection of immunized animals through the mucous membrane of the nose. Such an infection, as is known, leads to a more rapid invasion of the lungs with plague microbes than does the subcutaneous route.

An animal which has been weakly immunized (with weak vaccine or an inadequate dose of live vaccine) can stand up to infection subcutaneously and dies from infection through the nose. The observations of Bazarov on the greater changes in the lungs of immunized animals compared with the controls finds its explanation in the fact that the killed, hardly effective vaccine does not protect the animals from fatal plague but creates an incomplete immunity which is expressed only as a greater or lesser prolongation of the life of the animal compared with the controls. For this reason, the body of the animal which has been immunized with the hardly effective vaccine, resists for a longer time, and more marked involvements manage to develop in the organs (form of protective reaction of the body) than in the controls.

Such a reaction is particularly clear when weakly immunized animals are infected with massive doses of a virulent culture.

What has been said does not detract from the question of

the greater sensitivity of the immunized animals (guinea pigs) to pulmonary infection (by the aerogenic route) than to the subcutaneous route. This fact serves as the basis of the belief that the lung is more difficult to immunize than the other organs. The great sensitivity of pulmonary tissue to plague infection has stimulated certain investigators to seek other methods of immunizing against pulmonary plague.

Nicole, Duran and Conseillo
(1950) attempted to accomplish in practice the immunization of people through the respiratory tracts by the pulverization of a sufficiently dense (3 billion) killed vaccine for a half-minute a day for eight days; after a week's interruption, the inhalations were repeated for another eight days.

By this means a total of 363 persons were inoculated. As controls, 503 persons were inoculated subcutaneously. Among the latter, six persons became ill with plague, (five died); among those inoculated by the respiratory tracts, three became ill (two died). The data presented, understandably, do not permit any evaluation of the greater effectiveness of vaccinations by the inhalation method.

M. P. Pokrovskaya proposed combining subcutaneous immunization with repeated vaccinations by the respiratory route, the mucosae of the nose, eye, oral cavity, by the method of inhalation (up to six times) with a suspension of the live culture.

From the experiments of M. P. Pokrovskaya and L. S. Kaganov it was possible to draw the conclusion that neither a single subcutaneous inoculation nor even 4 inhalation vaccinations save all the animals from pulmonary plague infection.

However, by means of three subcutaneous inoculations it was, nevertheless, possible to protect 85.7 percent of the animals, while the four inhalation vaccinations protect only 66.7 percent of the guinea pigs from infection by pulmonary plague.

In further investigations for increasing the effectiveness of the live EB vaccine, Ye. I. Korobkova showed that the resistance of the body to pulmonary plague may be considerably increased by using for this purpose more active vaccinal strains and double vaccinations.

*p. 29 complete per
Mrs. Ethel Guttinger
H. Schick, Md.*

The significance of the repeated vaccinations with live vaccine is particularly clearly demonstrated in experiments for protecting against primary pulmonary plague.

The perspectives of future increase of effectiveness of live anti-plague vaccine are connected with the selection of highly active vaccine strains and the development of the problems of the most efficient form of using it. As a result of the experimental study of percutaneous and particularly of intracutaneous methods of immunization by live vaccine, new methods have been planned for the successful resolution of a number of problems associated with protection against pulmonary plague.

There is no doubt of the fact that as a result of immunization with effective live vaccine and inadequate dosage, the entire body as a whole--all the cellular systems and organs--acquire resistance to plague infection. In view of this, the viewpoint that it is impossible to protect the body from pulmonary plague by the percutaneous, subcutaneous and intracutaneous methods of inoculation and the need for administering the vaccine directly into the respiratory tracts does not appear to be convincing.

Such a hopeless view was created on the basis of utilization of hardly effective vaccines. The mechanism of immunity against pulmonary plague is no different from the mechanism of immunity to the bubonic form of plague. The difficulties of immunization against the pulmonary form of plague are conditioned by the fact that the plague process is here embracing a vitally important organ which is highly sensitive to the plague microbe and to its toxin. Judging by the observations of laboratory infections, solitary microbes which have entered the respiratory tracts can produce pulmonary plague in man (D. K. Zabolotniy, G. S. Kulesha, Wu Lien-Te).

To surmount this great degree of sensitivity, an effective live vaccine must be used which has been properly dosaged and properly applied. These statements are confirmed by the epidemiological observations in South Africa of the successful protection of people, inoculated subcutaneously by live vaccine, against primary pulmonary plague (see Chapter IV).

M. M. Faybich, by a comparative study of the immunogenic properties of the EB, M No 74, and the Otten Tzhivaydezh strains, found that the EB strain possesses the greatest

immunogenic properties; the M No 74 strain was more immunogenic than the Otten Tzhivaydezh strain. The weak immunogenicity of the latter strain is apparently conditioned by the unsatisfactory results of protecting against pulmonary plague which were noted by Otten in Java. It is hardly possible to dispute the idea that for the protection against pulmonary plague an immunity of greater intensity must be created than for protecting against bubonic plague.

However, there are no adequate proofs to the effect that vaccinations with the live vaccine directly into the respiratory tracts have any advantage over the other methods of inoculation. Immunization through the nose or by the inhalation method in the form that has been proposed by Nicole, Duran and Conseille and M. P. Pokrovskaya and L. S. Maganova gives weaker protection to guinea pigs against pulmonary plague than do the subcutaneous, percutaneous and particularly intracutaneous inoculations.

The conception of A.M. Bezredka of local immunity and the expediency arising from it of immunizing the organs most sensitive to the given infection (local vaccination) has proved to be worthless. Local use of cholera and typhus antigens (through the mouth) produces the appearance of antibodies in the sera of those who have received these antigens (Ye. I. Korobkova and Zenin, Glukhov and others).

Thus, local immunity is connected with general immunity. It is true, the mechanism of immunity to plague does not exhaust the formation of antibodies. At the same time there are no bases to deny the expediency of nasal or inhalational methods of immunization, which may be utilized as one of the methods of vaccination against bubonic and pulmonary plague. The latter experiments, conducted by us with highly immunogenic strains, confirm this attitude.

Thus, to the question of whether it is possible to protect against pulmonary plague with live vaccine an affirmative answer may be given.

The source of the skepticism of certain authors is the incorrect theory based on the utilization of inactive vaccines which protect only weakly against the pulmonary form of plague as well as against the bubonic form.

The successful solution of the problem of protecting against primary pulmonary plague should be sought in a systematic study of the active vaccinal strains, proper

dosage, and proper timing and routes of vaccination.

In the immunoprophylaxis of plague and in the protection against all the clinical forms of plague a most important condition is, in addition to using highly active injectable preparations for the vaccinations, an increase of the immunological reactivity of the macroorganism. Naturally, the dose effective for protecting guinea pigs cannot assure the protection of man from the pulmonary form of plague. The dose effective for protecting guinea pigs cannot assure the protection of man from the pulmonary form of plague. The dosage of vaccine should be computed from the minimal immunizing doses for guinea pigs and correspond to the effectiveness sought for man. The immunogenicity of plague vaccine, which may be determined on susceptible animals, coincides with its actual effectiveness in people.

The minimal immunizing dose of certain subcultures of the vaccine EB strain has at present increased to such an extent that for producing an immunity in a guinea pig doses have to be administered which are not much less than those for man (0.5-1 billion). Only with such a dose is it possible to protect 100 percent of all the animals used in the experiment from infection by 400-800 lethal doses of virulent plague culture.

At the present time, reactive changes in the spleen following administration of the EB culture, which were mentioned by Girard and Robic, are not found even after the administration intraperitoneally of such EB doses as 7-8 billion microbes. Because of this, the question arises as to the need for isolating new vaccinal strains, the minimal immunizing dose of which would equal hundreds of microbes and the need for reexamination of the dosage of the vaccine for human vaccinations. At the same time, the methods of inoculation should be studied taking into consideration the modern concepts of the immunological reactivity of the body and its significance in the problem of immunity.

CHAPTER XV

THE ALLERGIC SKIN REACTION AS AN INDEX OF IMMUNITY IN PLAGUE

Up to the present time, no reliable test exists for characterizing the specific changes of reactivity of the body which set in after the inoculations of live anti-plague vaccine. Single and even double vaccinations are not accompanied by notable accumulations of antibodies on the basis of which it might be possible to evaluate the immunological rearrangement of the body. In the serum of people and animals inoculated with live vaccine, no agglutinins, precipitins, bacteriolysins or other immune bodies are found which might attest to the onset of immunity, despite the fact that the animals immunized with the live vaccine acquire a great degree of resistance to the plague infection and tolerate the infection by large doses of the virulent microbes.

Thus, the protection of the body vaccinated against the plague is accomplished without the participation of the humoral factors of immunity which can be established by the usual serological methods. The role of the cellular phagocytic factors have been inadequately clarified.

As has already been mentioned, according to the data of M. P. Pokrovskaya and L. S. Kaganova, the index of anti-plague immunity which sets in after inoculations with live vaccine is the increase of the function of the cells of the reticulo-endothelial system. The processes which occur in the cellular elements of the inoculated body are characterized by the authors as a polyblastic reaction which passes into a stormy reaction on the part of the macrophages. The reaction is accompanied by the hyperplasia of the elements of the reticulo-endothelial system. The appearance of the macrophagic reaction coincides in time with the development of immunity. However, the extensive utilization of the reaction mentioned for practical purposes presents certain difficulties, since specially qualified personnel are required for reading of the cytozgrams.

With the aim of elucidating the possibility of utilizing an intracutaneous test for determining the immunological shifts in the body of those inoculated by live vaccine, investigations were conducted on changes of the skin reactivity in them.

A known capacity of the skin in many infections which regularly reflect the immunological and allergic rearrangement of

the body by its sensitivity to the injection of specific antigens served as the premises for the present investigations.

As is well known, at various periods in many diseases during convalescence and also after inoculations, the skin reactivity with respect to the specific causal agent is changed, and thereby in some cases the skin sensitivity is increased to the corresponding antigen; in others, it is decreased. The occurrence of an immunological rearrangement of the body can often be judged by the development of an increased sensitivity to the causal agent or to its products which is detectable by means of an intracutaneous test or percutaneous tests. Numerous investigations of natural positive cutaneous reactions show that they belong to the reactions of an allergic character. The allergen sensitizes the body only with respect to the repeated action of the same factor.

Any infection is to some degree accompanied by a sensitization of the body. The mechanism of this sensitization represents the result of the spread of allergizing substances (allergens) in the blood stream and their adsorption by all the tissues.

Allergic reactions and their significance in the pathogenesis and immunity to plague have been very little studied. Attempts of Amako to use the allergic tests for the diagnosis of plague have not been successful. In the opinion of N. N. Zukov-Verezhnikov, the fact itself of an increase of sensitivity in convalescents can be considered established.

V. N. Lobanov mentioned the presence in plague of characteristic allergic features, namely: rapid development and profound anatomical changes proper to plague, markedly manifested necrotic processes and constant presence of hemorrhages, and, finally, changes in the vascular system characteristic of allergy, particularly the phenomena of fibrinoid necrosis.

The disease plague in essence does not belong to the disease of allergic character; in other words, in the occurrence of plague the state of sensitization to the plague microbe is not a factor which pathogenetically determines the infection, but in the course of the disease sensitization occurs, in consequence of which allergic changes appear which complicate markedly the basic reactive process, transforming it in the direction of hyperergy.

The mechanism of development of increased sensitivity of the body from the point of view of the general physiology of exci-

tation is defined by Ado as a process of weak subthreshold stimulatory actions of a sensitizing antigen on the body at long time intervals.

The most important expression of allergy is the state of increased sensitivity.

Sensitization of the body which occurs in consequence of the injection of very small doses of the antigen without securing an adequate level of immunity should be distinguished from the allergic reaction of the infectious allergy type, which is the index of immunity and which accompanies it. This reaction occurs after having suffered from the disease as well as during the process of sickness with such infections in which the immunity bears the temporary or permanent character of a non-sterile immunity.

As antigen for the cutaneous reaction, killed vaccine made of plague microbes of the KB strain which had been grown on simple agar was used.

The antigen represented a suspension of a two-day agar culture in physiological saline solution, killed by keeping at 60°C for an hour. After a control was made for sterility, the culture was diluted to the required concentration and used for the intracutaneous test. By analogy with tularin, this allergen was called pestin.

The question of the interrelationship of the cutaneous allergy and immunity in plague was studied on guinea pigs known to be immune, which had survived an infection by virulent microbes, and on animals which had been immunized before they were infected. For the intracutaneous tests, doses of 20-25 million microbes per 0.1 cc of physiological saline solution were used. Such a dose of pestin gave the most constant results.

Experiments which were conducted in large numbers of experimental animals showed that in guinea pigs which were immune to plague, which had survived a control infection by a virulent culture of the plague microbe, an allergic rearrangement of the body occurs together with the immunological rearrangement. The latter appears as increased skin reactivity to the injection of plague allergen--pestin.

The reaction to pestin in immune animals usually appears 10-18 hours after the injection in the form of a more or less distinct erythema of the skin, an infiltrate, and the formation

at the site of the injection of a necrosis or ulcerative defect of the skin.

All these phenomena are maintained for two to three days, after which they begin to abate, and at the site of the injection a small node remains or a crust forms, which then falls off. Control, fresh guinea pigs do not react to the injection of pestin, or, in rare cases, a reaction is observed in solitary animals, which is notably different from the reaction which develops in the immune animals (Table 19).

Table 19

Intracutaneous test with immune guinea pigs				
Number of pigs	Evaluation of Reaction			
	acute	clear	weak	negative
24 immune	10	4	-	-
14 control	-	-	1	13

The allergic intracutaneous test is an index of the immune state of the body, and it apparently can be utilized for the detection of the effectiveness of the inoculations with live vaccine. This attitude has found its confirmation in the experiment reflected in table 20. In the great majority of vaccinated guinea pigs, a well expressed change of skin reaction to pestin corresponds to a strong immunity.

Table 20

Intracutaneous test with guinea pigs immunized against plague with one billion microbes of live vaccine subcutaneously

When intracutaneous test was made	Number of animals	Evaluation of reaction				Results of infection with 200 DLM		
		acute	clear	weak	negative	Number of pigs	Died	Survived
Within 20 days after vaccination	15	8	6	1	-	15	0	15
control	10	-	-	-	10	10	10	0

The intracutaneous tests, performed on different days after the immunization showed that in the first four to five days

after the inoculations the reaction in the great portion of the animals is negative; only after the 6th day does it become positive. This time coincides with the development of immunity in the animals, which is found by infecting them with a virulent culture of the plague microbe. With two inoculations, the positive intracutaneous reaction is found 24 hours after the second inoculation. The method of immunization with live vaccine is reflected on the results of the intracutaneous test.

The guinea pigs were immunized subcutaneously, percutaneously and intracutaneously. Beginning with 24 hours after the inoculation, the reaction to pestin was determined in a part of the guinea pigs of each group. These experiments showed that in the guinea pigs which were inoculated percutaneously and intracutaneously, the allergic rearrangement of the body is accomplished more rapidly and the reaction itself proceeds somewhat more expressively than in those guinea pigs which were subcutaneously inoculated.

Animals which had suffered from the plague react to pestin negatively (negative allergy). The positive intracutaneous reaction in those which became sick is a favorable prognostic feature and indicates the active fight of the body against the infection.

The immunized guinea pigs react markedly to the intracutaneous injection of the allergen following their infection with virulent culture, beginning with the first day. Such a positive intracutaneous test in the immunized animals is a good prognostic feature. A negative reaction indicates the absence of resistance of the body to the infection. Numerous observations made in the course of the current experiments showed the correctness of this conclusion and the prognostic significance of the intracutaneous tests.

The experiments performed showed that the intracutaneous test with pestin can be successfully utilized for the retrospective diagnosis of plague. In animals which have survived after a control infection of them with virulent microbes the positive reaction to the intracutaneous injection of the allergen is preserved up to six months, and this is not the limit. At present, in our laboratory L. P. Pavlova, a post-graduate student, has developed and tested on animals a new preparation of pestin which is highly sensitive and specific.

An attempt was made to test the skin reaction to pestin in volunteers who had been inoculated against plague. As antigen, a suspension of 100 million microbes in physiological saline

solution of a two-day agar, heated-killed EB culture was used. One-tenth cc of the antigen was injected into the skin of the middle third of the volar surface of the forearm.

In total, the intracutaneous reaction was tried out on 15 volunteers who were vaccinated one, two, three and five months before the given test was performed. One of them was inoculated nine months and another 11 months before the present experiment. In all, 15 volunteers the intracutaneous test was positive.

The reaction develops quickly--as early as six-seven hours after the injection inflammatory signs appear: marked erythema of the skin, small amount of edema, mild pain. In the center of the hyperemic area of skin a more darkly stained papule is clearly visible. The dimensions of the hyperemic area of skin reached, on the average, 3.5 x 3.5 c. In 24 hours the reaction begins to abate and disappears altogether after three-four days. Only in one volunteer was the reaction more marked. Around the site of the injection an area of hyperemia was formed with dimensions of 5 x 3.5 cm with mild pain there. The reaction was maintained up to six days. It should be mentioned that this colleague had been inoculated with live vaccine more than once, and the last inoculation had left a deep scar on his arm with a tissue defect.

There is basis to believe that the great reactivity of the skin to pestin may correspond to a more intense immunity, developing after inoculations. With respect to animals, this statement has been proved.

After the intracutaneous test, a small thickening remains in the skin at the site of the injection as well as an erythema which disappears after five-six days. In those which had not been inoculated, the test with pestin was negative. Only a traumatic reaction was noted, a limited hyperemia without an infiltrate. After a day, the redness began to lessen.

Testing of the intracutaneous reaction on people, it is understood, still cannot be considered perfect and entirely convincing. In order to draw a conclusion as to the significance of the indicated reaction for practical purposes, the intracutaneous tests need to be studied on a large number of inoculated persons.

Apart from this, the necessity can come up of differentiating reactions of the type of infectious allergy from the reaction of increased sensitivity to repeated administrations

of the allergen itself.

It has been established that with repeated intracutaneous tests performed on non-immunized guinea pigs, the latter begin to react positively to the pestin. It is true, the reaction proceeds somewhat more weakly in them than in the immune guinea pigs, but it is nevertheless clear. Infection of such guinea pigs with virulent microbes has shown that as a result of repeated intracutaneous injections of pestin, a certain resistance to plague develops in them, which appears as a prolongation of their lives compared with the control animals.

It may be supposed that the reactions under study on the guinea pigs known to be immune and those which have been immunized, in which the state of immunity has been checked with subsequent infections of them with virulent cultures, present a clear-cut example of the unity of the allergic state and the state of immunity in the body.

In conclusion, it should be noted that the thesis established in experiments to the effect that an allergic rearrangement of the body occurs in immunized animals, which rearrangement can be detected by an intracutaneous test, is promising for clinical practice. The intracutaneous tests can be utilized: 1) for determining the immunological rearrangement of the body, 2) for retrospective diagnosis of plague.

END